1. PURPOSE: To define the procedure to quantify the total amount of amplifiable human male DNA in a sample using Quantifiler™ Y Human Male DNA Quantification Kit (Quantifiler Y).

2. SCOPE: The Quantifiler Y Kit will be used by the NCSBI Forensic Biology Section to quantify the amount of human male DNA extracted from forensic evidence samples.

3. SAFETY

   3.1. Quantifiler Y PCR Reaction Mix may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

   3.2. During the operation of the thermal cycler, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Keep hands away from the heated cover and sample block.

4. DEFINITIONS

   Y-STRs: Short Tandem Repeat sequences from the male-specific Y-chromosome.

5. REFERENCE DOCUMENTS

Applied Biosystems ABI Prism® 3100 Analyzer and 3100-Avant Genetic Analyzer User Reference Guide.

Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide (P/N 4352715).

Applied Biosystems 3130/3130xl Maintenance, Troubleshooting, and Reference Guide (P/N 4352716).

Applied Biosystems. ABI Prism® Genetic 3100 and 3100-Avant Genetic Analyzers – Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products. User Bulletin. 2003.

Applied Biosystems. Quantifiler™ Kits: Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual. 2003.

Butler, J.M. Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

"Developmental Validation of the Quantifiler™ Real-Time PCR Kits for the Quantitation of Human Nuclear DNA Samples." J Forensic Sci, July 2005, Vol. 50, No. 4. Robert L. Green, B.A.; Ines C. Roinestad, MS; Cherisse Boland, B.A.; and Lori K. Hennessy, Ph.D.

NCSBI Validation Studies for Y-Filer and Quantifiler Y.

6. PROCEDURE

6.1 Preparation of Standards

6.1.1 Label eight microcentrifuge tubes: Std. 1, Std. 2, Std. 3, and so on. Thaw the PCR reaction mix and primer set, then vortex 3 to 5 seconds and centrifuge briefly before opening the tubes.

6.1.2 Dispense the required amount of glycogen:TE to each tube.

6.1.3 Prepare Standard 1:

6.1.3.1 Vortex the Quantifiler Y Human DNA Standard for 3 to 5 seconds and centrifuge briefly.

6.1.3.2 Using a new pipette tip, add the calculated amount of Quantifiler Y Human DNA Standard to the tube for Std. 1.

6.1.3.3 Mix the dilution thoroughly.

6.1.4 Prepare Standard 2 through 8:

6.1.4.1 Using a new pipette tip, add the calculated amount of the prepared standard to the tube for the next standard.

6.1.4.2 Mix the standard thoroughly

6.1.4.3 Repeat steps 6.1.4.1 and 6.1.4.2 until the dilution series is complete.

Standard	Concentration (ng/µL)	Example Amounts	Dilution Factor
Std. 1	50.000	50 µL stock + 150 µL TE Buffer	4X
Std. 2	16.700	50 µL Std. 1 + 100 µL TE Buffer	3X
Std. 3	5.560	50 µL Std. 2 + 100 µL TE Buffer	3X
Std. 4	1.850	50 µL Std. 3 + 100 µL TE Buffer	3X
Std. 5	0.620	50 µL Std. 4 + 100 µL TE Buffer	3X
Std. 6	0.210	50 µL Std. 5 + 100 µL TE Buffer	3X
Std. 7	0.068	50 µL Std. 6 + 100 µL TE Buffer	3X
Std. 8	0.023	50 µL Std. 7 + 100 µL TE Buffer	3X

6.2 Setting up Quantifiler Reactions

6.2.1 Calculate the amount of reagents needed for the number of samples you are analyzing by using the appropriate Quantitation Setup Worksheet. **Note:** If setting up samples for both human and Yfiler quantitation on the same plate, the user must differentiate human vs "Y" by using different names for each sample.

6.2.2 Save the sample setup by copying and pasting into a notepad file and saving the file on the CODIS Laptop associated with the instrument that you will be using.

6.2.3 Reagent Preparation

6.2.3.1 Thaw the Quantifiler Y primer mix completely, then vortex 3-5 seconds and centrifuge briefly before opening the tube.

6.2.3.2 Swirl the Quantifiler™ Y PCR Reaction Mix gently before using. Do not vortex.

6.2.4 Pipette the required volumes of components into an appropriately sized polypropylene tube.

6.2.5 Vortex the PCR master mix for 3 to 5 seconds, then centrifuge briefly.

6.2.6 Dispense 23 µL of the PCR master mix into each reaction well.

6.2.7 Add 2 µL of sample, standard, or control to the appropriate wells. The final reaction volume should be 25 µL. For the NTC (negative control) add 2 µl of TE buffer.

6.2.8 Seal the reaction plate with the optical adhesive cover. Use plastic tool to seal optical cover in place. Tear off white edges.

6.2.9 Centrifuge the plate at approximately 2000 rpm for about 2 minutes in a tabletop centrifuge with plate holders.

6.2.10 If you are using a 7000 instrument, place the compression pad over the Optical Adhesive Cover with the gray side down and the brown side up and with the holes positioned directly over the reaction wells. **IMPORTANT!** Do not use a compression pad if you are using a 7500 instrument.

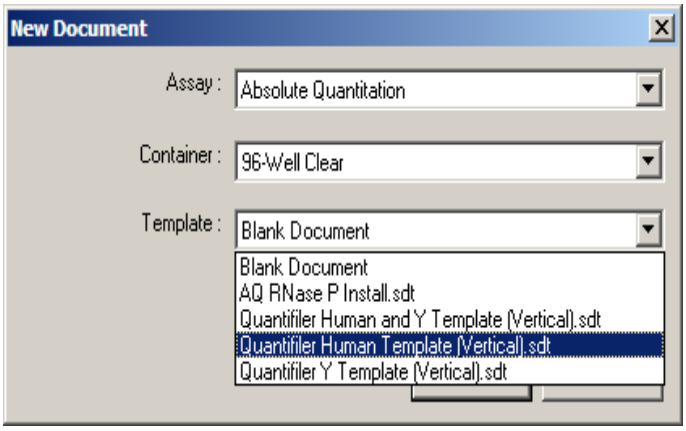
6.3 Setting up a Plate Document

6.3.1 Turn on the ABI PRISM® 7000.

6.3.2 Open up ABI PRISM® 7000 SDS Software v1.0 or most recent version.

6.3.3 Click "File" and choose "New".

6.3.3.1 A box will appear in the middle of the screen that looks like this:

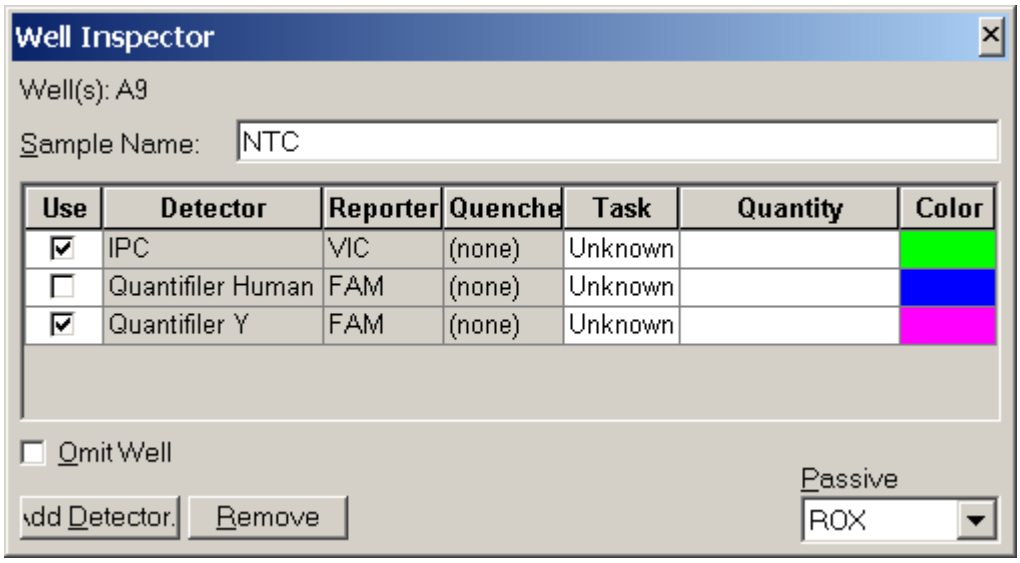


6.3.3.2 Select the appropriate template for your reactions.

6.3.3.3 Click on File in the menu bar and click on Import Sample Setup

6.3.3.4 Choose the file you saved on that computer from step 6.2.2

6.3.3.5 Click on View in the menu bar and click on Well Inspector. Highlight all the wells that you are not using and click the “Omit Well” box in the Well Inspector (optional).



6.3.4 After completing the plate setup, click on the Instrument tab and check the settings. The settings should look like this:

	Stage 1	Stage 2	
# of cycles (Reps)	1	40	
Temp. (Celsius)	95	95	60
Time	10 minutes	15 seconds	1 minute

6.3.5 Also make sure that the sample volume is set to 25 µl and that the box next to "9600 Emulation" is checked

6.3.6 Click File on the menu bar and click Save as to save and name your document (this must be done before the machine will start collecting data).

6.4 Running the Reactions (7000 SDS)

6.4.1 Lift the handle at the bottom of the door on the front of the instrument until the door is raised completely. Gently push the carriage back until it stops and locks into place.

6.4.2 Position the plate in the instrument thermal block so that:

- Well A1 is in the upper-left corner
- The notched corner of the plate is in the upper-right corner

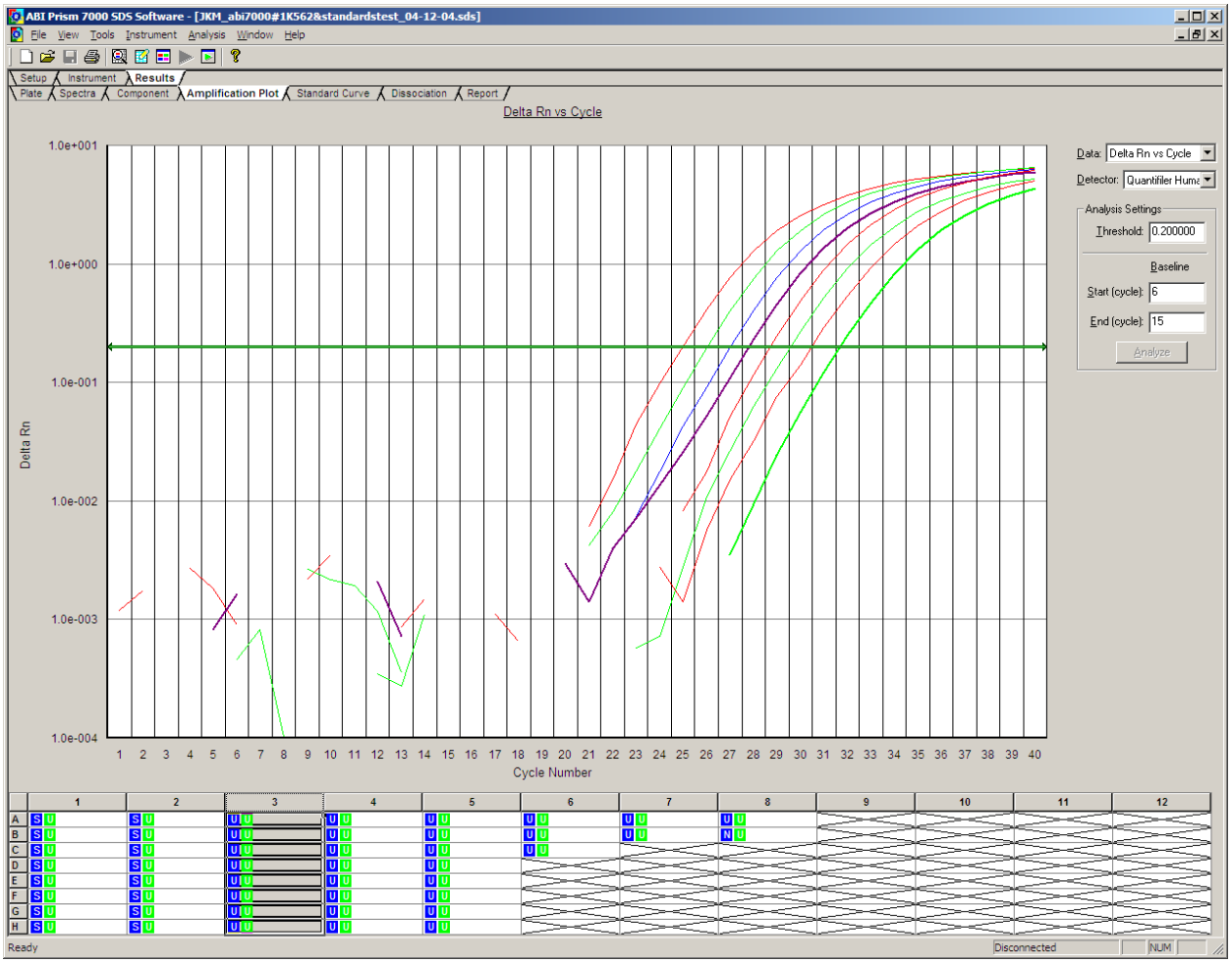
6.4.3 Gently push then release the carriage to unlatch it. The carriage automatically slides forward into position over the sample plate. Load the plate into the thermal cycler. After the door moves to the front, pull the handle down to close the cover gently. In the SDS software, open the plate document that you set up for the run.

6.4.4 Select the **Instrument** tab, and then click **Start**.

6.5 Analyzing Data in SDS Software

6.5.1 When the ABI PRISM® 7000 run is finished, click on the Analyze button (green arrow in the tool bar).

6.5.2 Click the Results tab and highlight all the wells to see your results. This will show your quantity results in a plate format.

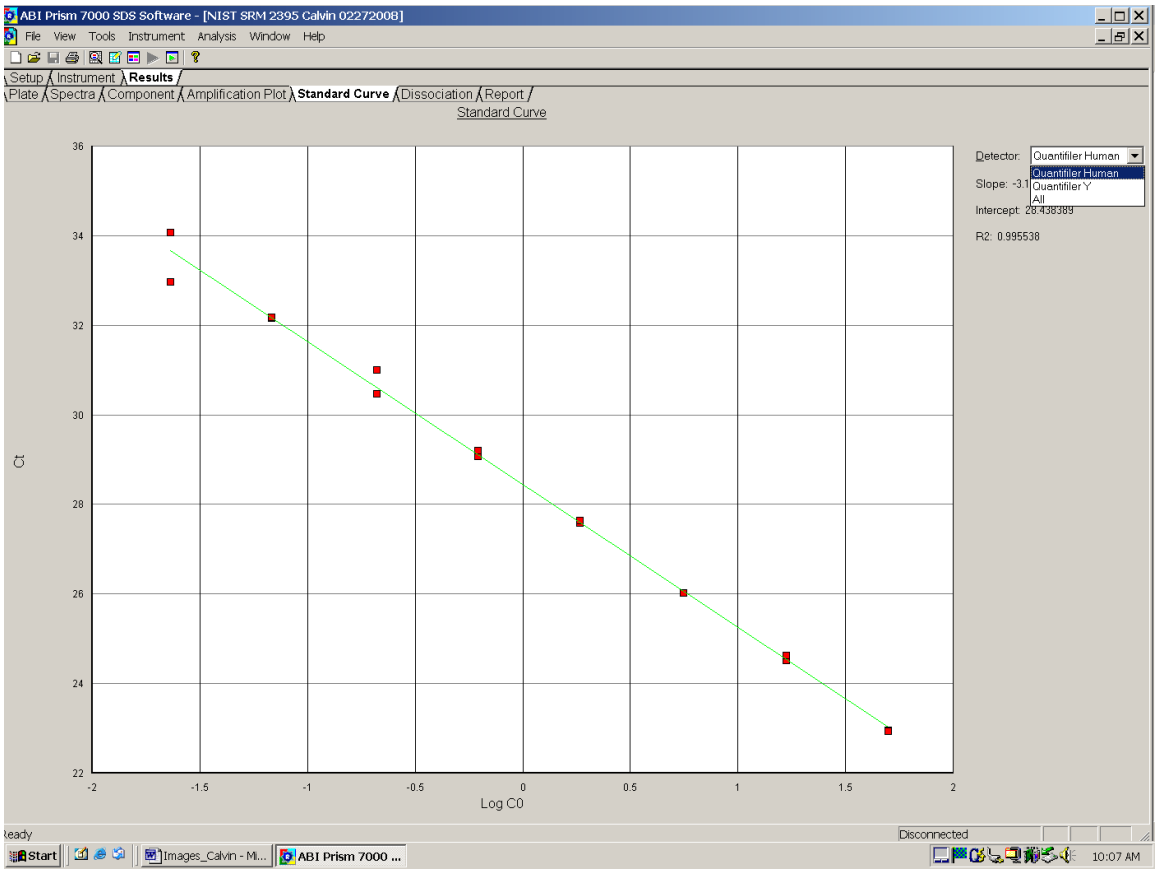


6.5.3 Click on the Amplification Plot tab and check all of your data to make sure there is no unacceptable activity in the negative control and that the internal positive control (IPC) reaction worked in each well.

6.5.4 Standard Curve

6.5.4.1 Click on the Standard Curve tab.

6.5.4.2 In the Detector drop-down list, select the detector that corresponds to the kit you are using.

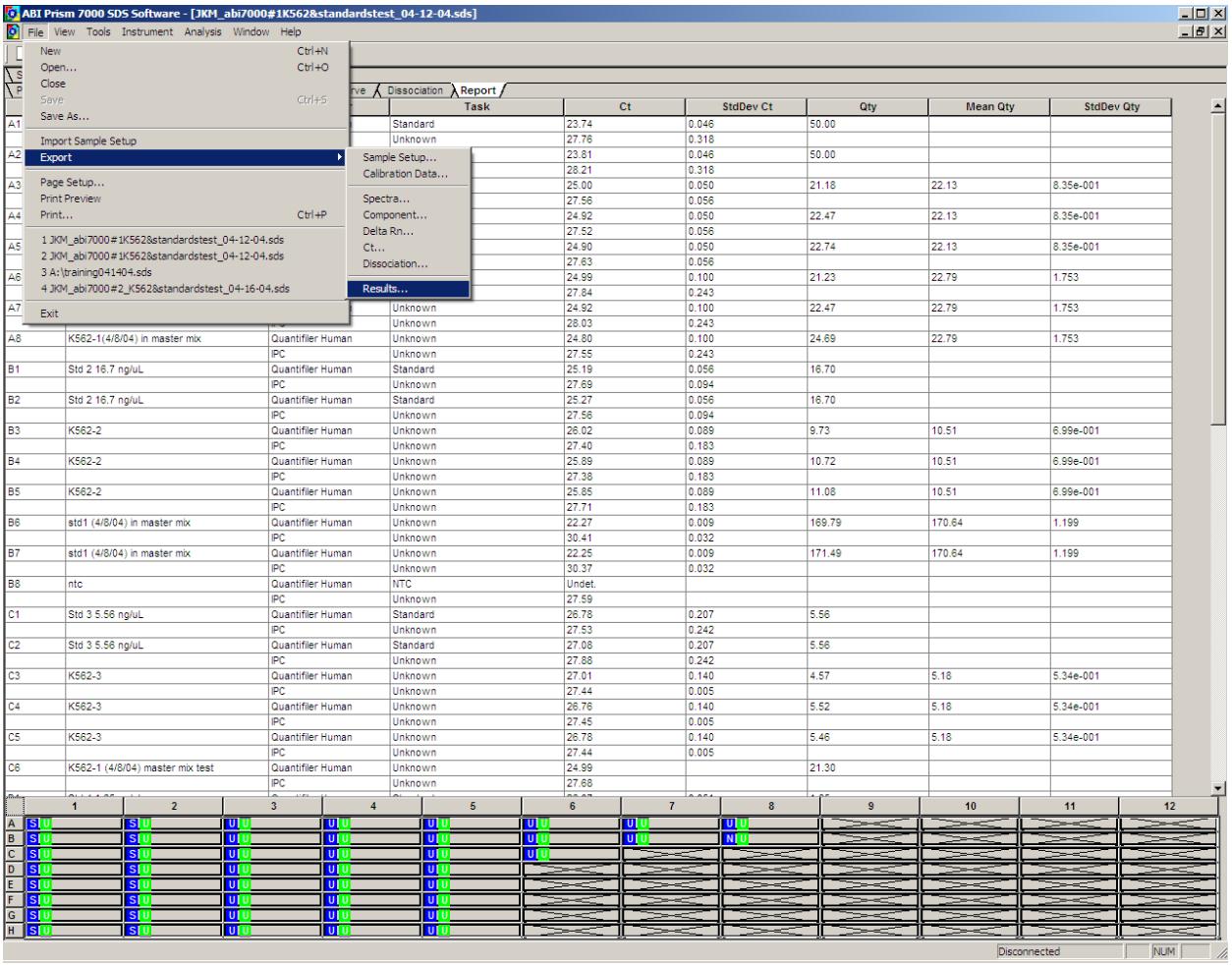


6.5.4.3 Check the values of your slope and R^2. The slope should be between -3.0 and -3.6, while the R^2 value should be greater that 0.98. If the value falls outside of this range, then one point of the slope may be dropped to account for pipetting variations. If a point is dropped, then both the original and adjusted slopes must be printed and placed in the case notes. The intercept indicates what the Ct value of a 1ng/µl sample would be for that run.

6.5.4.4 Click on the “Report” tab.

6.5.4.5 Click the “File” button, go to “Export”, and click “Results”.

6.5.4.6 Save the file in your folder on the computer.



6.5.5 Analyzing data in Microsoft Excel

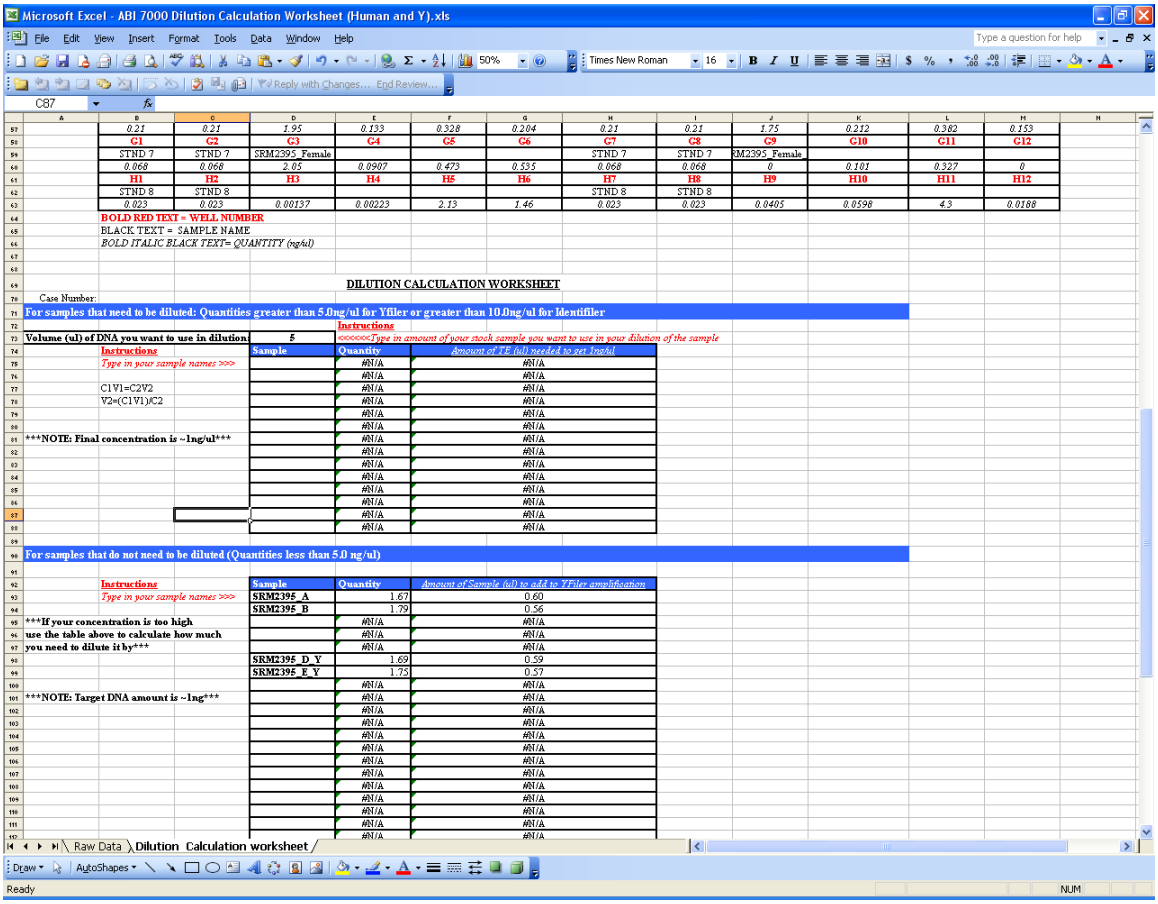
6.5.5.1 Open the exported results document.

6.5.5.2 Open the appropriate ABI 7000 Dilution Calculation Worksheet.

6.5.5.3 Select all the data on your results document by pressing Ctrl + A.

6.5.5.4 Click on cell A1 of the “Raw Data” section of the ABI 7000 Dilution Calculation Worksheet and paste your results.

6.5.5.5 Click on the “Dilution Calculation Worksheet” tab and you will see your results in plate format.



6.5.5.6 Enter sample names in the spreadsheet according to whether you will need to dilute that sample or not. (There needs to be approximately 1ng of DNA in the Y-Filer reaction, therefore samples that are ~5 ng/µL and lower do not need to be diluted.) The spreadsheet will automatically calculate the amount of TE needed for dilution or the amount of DNA needed for the Y-Filer reaction.

6.5.5.7 Print out your results on the calculation worksheet and use it to prepare your samples for Y-Filer amplification reactions

7. APPENDICES

None

Revision History		
Effective Date	Revision Number	Reason
Revision History		
April 10, 2008	00	Original Document

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	